Night Monkey Hybrids Exhibit De Novo Genomic and Karyotypic Alterations: The First Such Case in Primates

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Abstract

Using molecular chromosomal analyses, we discovered night monkey hybrids produced in captivity from matings between a female *Aotus azarae boliviensis* ($2n = 50$) and a male *Aotus lemurinus griseimembra* ($2n = 53$). The parents produced seven offspring in total, including one male and six females—a pattern consistent with Haldane's rule. Chromosomal studies were conducted on four of the hybrid offspring. Two of them showed relatively "simple" mixture karyotypes, including different chromosome numbers ($2n = 51, 52$), which were formed because of a heteromorphic autosome pair in the father ($n = 26, 27$). The other two hybrid monkeys exhibited de novo genomic and karyotypic alterations. Detailed analysis of the alterations revealed that one individual carried a mixture karyotype of the two parental species and an X chromosome trisomy (53,XXX). The second individual displayed trisomy of chromosome 18 (52,XX,+18) and a reciprocal translocation between autosomes 21 and 23 (52,XX,+18,t(21;23)). Interestingly, the second monkey exhibited mosaicism among blood cells (mos52,XX,+18[87]/52,XX,+18,t(21;23)[85]), but only a single karyotype (52,XX,+18) in skin fibroblast cells. The X- and 18-trisomies were derived from a doubling of the mother's chromosomes in early embryonic cell division, and the reciprocal translocation likely developed in the bone marrow of the offspring, considering that it was observed only in blood cells. Such occurrence of trisomies in hybrid individuals is a unique finding in placental mammals.

Key words: interspecies hybridization, chromosome paint analysis, trisomy, reciprocal translocation, mosaicism, hybridization effect.

Introduction

Speciation mechanisms are the ultimate forces underlying the diversity of life on Earth (Darwin 1859). Among these are powerful macro-scale phenomena including chromosome alterations that involve large blocks of genomic information. Suggestive cases indicating an important evolutionary connection are demonstrated in earlier research and include chromosomal speciation, hybrid speciation, and dramatic genome changes in hybrids (O'Neill et al. 1998; Reiseberg et al. 1995; Hauffe and Searle 1992; Garagna et al. 1997). Interspecific or interracial hybridization can produce genomic stress via creation of "mixed" chromosomal elements that then become a significant source of genomic modification (McClintock 1984). Hybrid offspring often have such incongruent chromosomal components that can, for example, disrupt mechanisms for suppressing transposable elements (Carson 1990). Several studies have found evidence corroborating these notions of chromosomal stress/instability (e.g., Rieseberg 2001; Fontdevila 2005; Metacalfe et al. 2007; Ostberg et al. 2013). Interestingly, though chromosomal rearrangements have been observed in marsupial hybrids (O'Neill et al. 1998), similar rearrangements have not previously been seen in placental mammal hybrids (Roemer et al. 1999). Recently, however, we encountered for the first time de novo chromosomal alterations in hybrid individuals of night monkeys (also known as owl monkeys), a primate taxon of placental mammals.

Night monkeys are a distinct genus (*Aotus*) within the radiation of New World Monkeys (Platyrhinni). Eleven species are distributed in the northern part of South America, and their chromosome numbers (from 46 to 58) vary with geography (fig. 1) (see also Ruiz-Herrera et al. 2005; Menezes et al. 2010). Thus, karyotypes are important tools for identifying species and should be used to distinguish individuals in captivity (Ma 1981; de Boer 1982; Ford 1994; Groves 2001; Defler and Bueno 2007). Karyotype differences are excellent genetic markers for determining taxonomic identification and studying molecular evolutionary mechanisms. In primates,

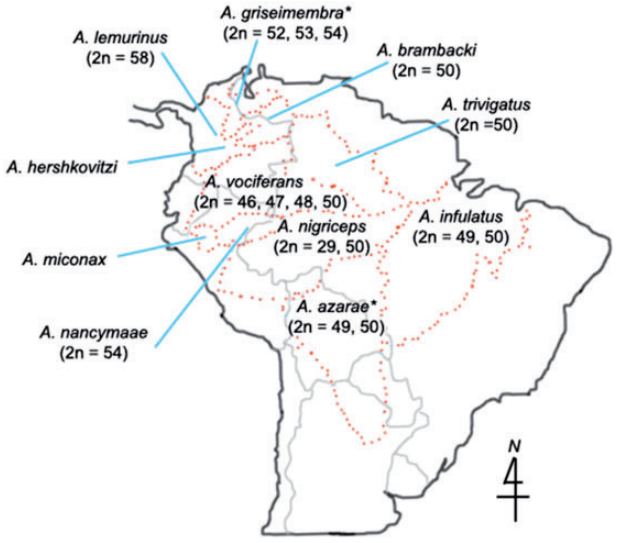

Fig. 1.—Geographic distribution of the genus *Aotus*, following Menezes *et al.* (2010) with modification based on Hershkovitz (1983). Chromosome numbers of each species are given in parentheses, with additional information from Ruiz-Herrera et al. (2005). Gray lines represent borders between countries, and red dotted lines represent borders of species distributions. Asterisk shows species used in the present study. *Aotus azarae* and *Aotus griseimembra* accord with *A. azarae boliviensis* (AAB) and *A. lemurinus griseimembra* (ALG), respectively, as described in the main text.

even closely related taxa often exhibit distinct karyotypes. However, the relationship of genome/chromosome differentiation and speciation is not fully understood. Hybridization has been proposed as a significant evolutionary mechanism that can result in speciation of primates (e.g., Tosi et al. 2000, 2003; Arnold and Meyer 2006).

In the 1970s, the Primate Research Institute, Kyoto University (KUPRI) acquired four night monkeys to establish a small breeding group. Two individuals were from Bolivia, and two other individuals were without provenance information. At the time of receipt, the KUPRI faculty believed they were all members of the same species (identified then as *Aotus trivirgatus*). They were housed as breeding pairs and produced many offspring. More recently, however, chromosome analysis revealed that the four founders represented two different species (Nagao et al. 2005). They included different chromosome numbers (Ruiz-Herrera et al. 2005): $2n = 49$♂/50♀ (*Aotus azarae boliviensis*: AAB), of which we received one male and two females, and $2n = 53$♂ (*Aotus lemurinus griseimembra*: ALG), of which we received one male. The odd numbers of male chromosomes are due to the fact that the Y-chromosome has inserted into autosome No. 14 in the former species (Ma 1981) and that an autosome was made heteromorphic (heterokaryotype) by Robertsonian rearrangement in the latter species (Ma 1981). These karyotype differences were not known in the 1970s, and the great morphological similarity among several *Aotus* species often led to misidentifications and the inadvertent creation of hybrids in captivity (Menezes et al. 2010).

In light of the new chromosomal data, we recognize that our four founders represented two different species (AAB and ALG) (Groves 2001; Ruiz-Herrera et al. 2005; Menezes et al. 2010), and that seven of the first generation (F1) offspring thus were hybrids (fig. 2). We examined the karyotypes of four of the F1 hybrids, and found that they indeed have mixed genomes consisting of two different karyotypes. Two individuals have "simple" mixed karyotypes that can be distinguished by the different chromosomal patterns of their parents, but the other two hybrids have more complicated karyotypes. For the latter two individuals, we conducted experiments using molecular cytogenetic techniques to better document their complicated patterns and found two trisomies, a reciprocal translocation, and the mosaic condition of these. The occurrence of trisomies in these hybrids is a unique finding in placental mammals, based on our review of the literature. The present study describes these karyotype findings and discusses derivation mechanisms of the de novo chromosome alterations that occurred in the hybrid offspring.

Materials and Methods

Animals

KUPRI records show that four night monkeys (two females and two males) arrived in 1971 and 1977 (fig. 2). Individual number 14 male (ID14M) and 23 female (ID23F) were unknown origin and ID24M and ID25F were Bolivian origin. The two females and two males were paired to produce the first KUPRI-born generation. Pairing of ID25F with ID24M produced seven offspring (four females and three males) from 1980 to 1986, and pairing of the same female with ID14M produced seven offspring (six females and one male) from 1986 to 1992 (fig. 2). A cytogenetic analysis revealed that the four founding night monkeys included two different karyotypes: $2n = 53$ (karyotype III of Ma et al. 1976) and $2n = 49$, 50 (karyotype VI of Ma et al. 1976). A separate study revealed that these different karyotypes indeed represent two species: AAB and ALG from different regions in South America (Nagao et al. 2005). ID23F and ID25F had a diploid chromosome number of $2n = 50$, and ID24M had $2n = 49$. All three individuals represent species AAB. ID14M, representing species ALG, had $2n = 53$ and a karyotype that exhibited a heterozygosity of a large metacentric chromosome and two midsized acrocentrics formed by either centric fission or centric fusion (Ma 1981). The offspring of ID25F and ID14M were therefore all interspecies hybrids. Information regarding the chromosome disorder of hybrids as a potential function of mother's age at breeding is presented in the pedigree chart of figure 2.

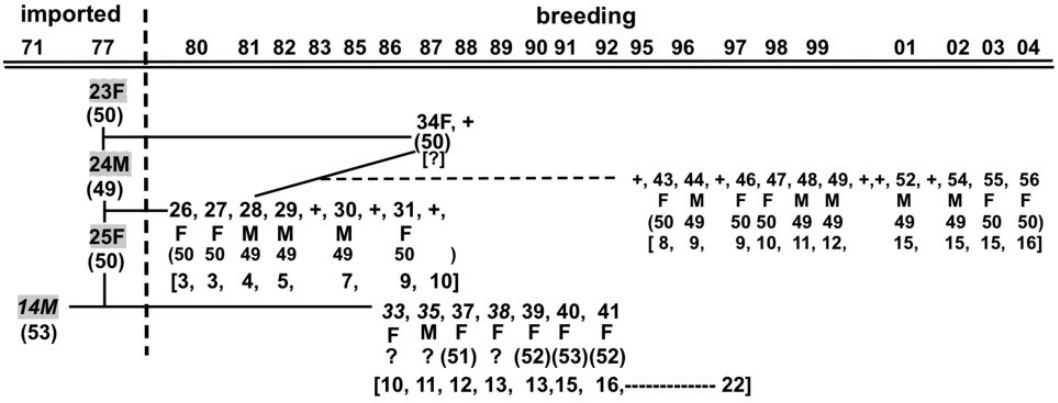

Fig. 2.—Partial pedigree chart of night monkeys that have been raised in KUPRI. Numbers in the top row indicate year of importation or breeding (e.g., 1971, 1977). Numbers in the next row indicate individual ID # and sex, male "M' vs. female "F" (e.g., 23F, 34F, 44M). Shadows highlight the IDs of the four founders, and italics denote the animals that died before this study was conducted. Numbers in parentheses indicate chromosome number for each individual. Numbers in the square bracket are the ages of the mothers (ID25F, ID34F) at the time of birth of each offspring. Founding mother 25F stopped breeding at 16-years-old and was 22-years-old at time of death. Plus (+) denotes a spontaneous abortion or stillbirth. Question mark (?) indicates lack of information.

Chromosome preparations treated with R-banding of ID14M were kindly supplied by Drs M. Hirai and S. Kawamura of The University of Tokyo, as the animal itself died years ago. All experiments were conducted according to the Guidelines for Care and Use of Non-human Primates of KUPRI (version 3; Primate Research Institute, Kyoto University 2010).

Chromosome Preparations and Chromosome Paint Analyses

To examine the chromosomes of the interspecies hybrids, we cultured blood cells and fibroblast cells of ear skin as described previously (Hirai et al. 1989; 2002). Chromosomes were arranged and described following previous numbering systems (Pieczarka and Nagamachi 1988; Stanyon et al. 2011). For a more detailed analysis, we then employed fluorescence *in situ* hybridization (FISH) (Hirai et al. 2007) with night monkey paint probes made from target chromosomes using a micro-dissection technique for a single scrape (Hirai et al. 2012). Briefly, we scraped individual chromosomes, or chromosomal segments, with a glass capillary of 2 μm diameter made using a puller machine (Narishige Tokyo, Japan). Spreads of chromosome preparations on a cover slip were used for scraping, and a scraped chromosome segment was subjected to Dop-PCR with a primer (5′-CCG ACT CGA CNN NNN NAT GTG G-3′) designed for chromosomal sequences. The specific DNA obtained as PCR product was labeled using haptens (digoxigenin-11-dUTP and biotin-16-dUTP, Roche) and used for paint analyses. Digoxigenin was detected with anti-Dig-antibody-FITC Fab fragments (green color paint) (Roche) and biotin was detected using Ultra Avidin (biotin affinity) Rhodamine (red color paint) (Leinco TGechnologics Inc.). Chromosomal DNA was denatured with alkaline solution (pH 12.5 in 2 × SSC for 4 min) and probe DNA was denatured by heating (at 70 °C for 5 min). Posthybridization washing was done by 40% formamide in 2 × SSC for 10 min at 45 °C, 2 × SSC for 10 min at 45 °C, and 2 × SSC for 10 min at room temperature. Before detecting hybridization, the slide was immersed in BI buffer and detection reagent was added as conjugate with BI buffer. Image uploading and analysis were done using IPLab/Mac of Scanalytics, Inc. (USA) (Solution Systems in Japan).

DAPI-banded (similar to G-banded) chromosomes that are formed by counterstaining using 4′,6-diamidino-2-phenylindole (DAPI) in FISH analysis were used to distinguish AAB and ALG chromosome patterns. To identify the X chromosome in the parent genomes, we performed FISH analysis with a tandem repeat sequence (OwlRep) probe specific for night monkeys (Prakhongcheep et al. 2013). The probe, a tandem repeat of a 187-bp sequence, was cloned in a fosmid vector. The 5.1-kb sequence is deposited in DDBJ under accession number AB746944. This probe may be used to differentiate AAB and ALG, because the former species has a positive site in the proximal region of X-chromosome, but the latter species does not (see Results). On the basis of known human/night monkey chromosome homologies (Ruiz-Herrera et al. 2005), a human chromosome 17 probe (Cambio, UK) was used to detect translocation between chromosomes 21 and 23. Description of chromosome alterations followed guidelines set forth in An International System for Human Cytogenetic Nomenclature (ISCN) (Shaffer et al. 2013).

To elucidate chromosome differentiations in the four hybrid individuals, we observed >50 of both blood and skin fibroblast cells in each individual. Mosaicism was studied across a total of 172 blood cells of ID41F hybrid.

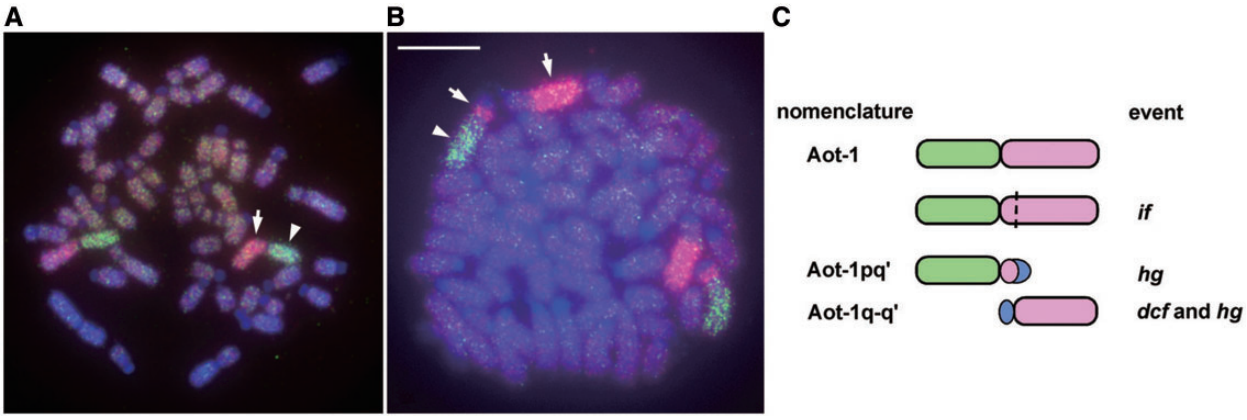

Fig. 3.—Chromosome paint analysis with probes of p (green) and q (red) arms produced from AAB chromosome 1 (Aot-1). (*A*) *Aotus azarae boliviensis* (ID25F) (AAB: Aot-1pq (red and green)). (*B*) *Aotus lemurinus griseimembra* (ID14M) (ALG: Aot-1pq′ (red and green) and Aot-1q-q′ (red)). Arrow shows Aot-1p (red) and arrowhead, Aot-1q (green). (*C*) Hypothesized rearrangement of Aot-1 showing chromosome nomenclature and sequential events in the change: *if*, interstitial fission. *hg*, heterochromatin growth. *dcf*, de novo centromere formation. Broken line, location of breakage. Scale bar = 10 μm.

Results

Birth Condition in Intraspecific and Interspecific Breedings

The database of our institute included information on the birth condition of our night monkeys. The relative values (%) of spontaneous abortion and stillbirth in the three mothers were as follows (see also fig. 2): ID34F had one spontaneous abortion (1 of 15 pregnancies, 7%) and four stillbirths (4 of 15 pregnancies, 27%) when mated with ID28M; ID25F had one stillbirth when mated with ID24F (3/9, 33%); ID23F had one stillbirth when mated with ID24M (1/2, 50%). Interestingly, seven interspecific hybrid offspring produced by mating of AAB-ID25F and ALG-ID14M did not show any disorder of birth (0/7, 0%), but offspring of intraspecific breeding resulted in a total of nine stillbirths (9/26, 34.6%) in the 26 pregnancies described above. Chromosome analysis was not performed for stillbirth offspring.

Representative and Informative Chromosomes of Parent Species

Night monkeys exhibit a wide range of karyotypes, which are thus informative for inferring cytogenetic phylogeny. As a first step, we identified chromosomes characteristic of each parent species (AAB and ALG). We focused on chromosomes 1 and 2 of AAB as they are easily distinguished from those of ALG and tentatively designated them Aot-1 and Aot-2, respectively. The largest chromosome in ALG (third largest in AAB karyotype) was designated Aot-3.

To analyze Aot-1 (the largest chromosome in AAB), we made distinct paint probes for the short arm (1p, green) and long arm (1q, red) in ID25F (mother of the hybrids) (fig. 3*A*). The homologous material in ID14M (father of the hybrids) was detected across four, rather than two, chromosomes (fig. 3*B*): a pair hybridized with green (1p) and red (partial q, 1q′) segments and a pair hybridized with only red (1q-q′). The most parsimonious interpretation is that these separate chromosomes resulted from an interstitial fission (*if*) in the q arm of Aot-1, heterochromatin growth (*hg*) of the short arm, and de novo centromere formation (*dcf*) (diagrammed in fig. 3*C*). Hence Aot-1 of the two species can be described as a single Aot-1 pair for AAB, but two separate pairs for ALG: Aot-1pq′ and Aot-1q-q′.

To analyze the second and third largest chromosomes, we began by making paint probes of the short arm (p, red) and the long arm (q, green) of the second largest chromosome (Aot-2) in ID25F (fig. 4*A* and *B*). The two paint probes showed existence of common DNA on both arms of Aot-2: there was a tiny band with yellow color on each arm that resulted from the mixture (yellow) of both colors, red and green (fig. 4*A*, white bars). This might indicate a pericentric inversion on the prototype of Aot-2 after entering metaphase, as will be described below. The two paint probes (p-red and q-green) made from Aot-2 also bound to the third largest chromosome, Aot-3, on the karyotype of ALG (fig. 4*C*). Again, the homologous material was found across two pairs of chromosomes in ALG: 1) a pair of small chromosomes that hybridized with red probe (Aot-2p), and 2) a pair consisting of one middle-sized acrocentric with the q arm hybridized with green probe (Aot-2q (acrocentric)), and one large-sized metacentric with the p arm hybridized with green probe (2q) and the q arm being nonhybridized (3q) (Aot-2q + nonhybridized arm = Aot-3) (fig. 4*C* and *D*). These differences are summarized in figure 4*I*, which shows the derivation mechanisms of the corresponding chromosomes. The derivation mechanisms for Aot-2 and -3 may be postulated from 2p and 2q that are regarded as prototypes. The combination of evidence

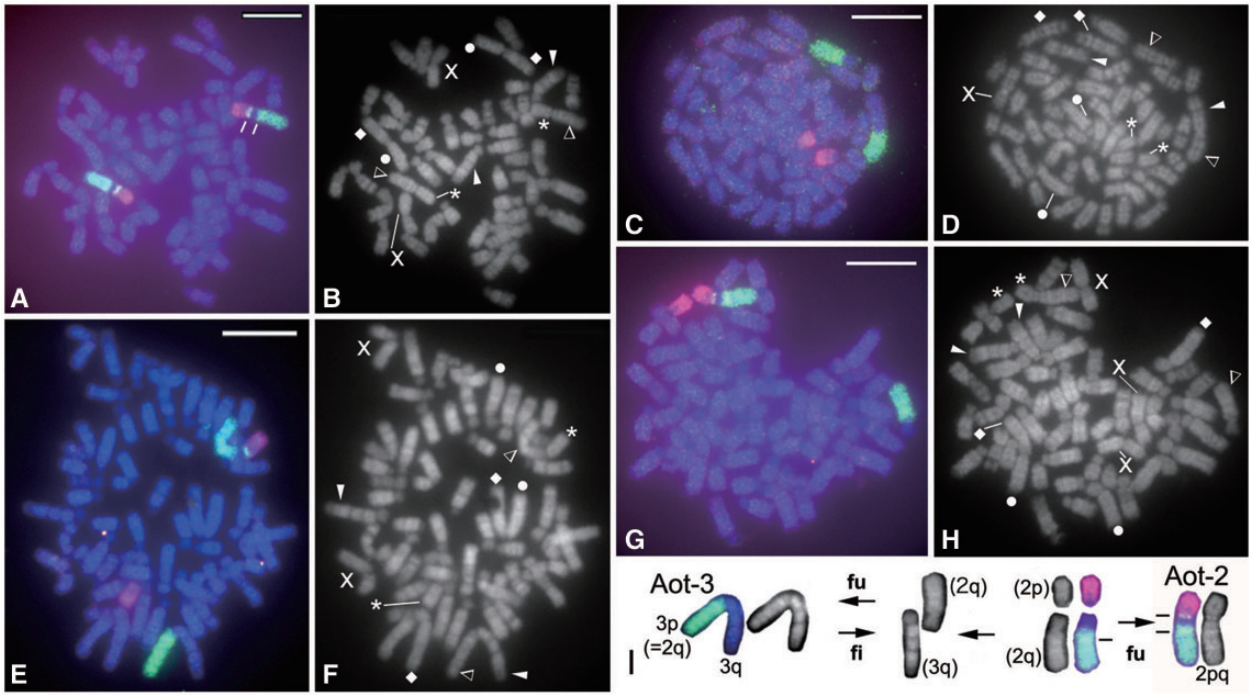

Fig. 4.—Chromosome paint analysis using probes of the short arm (p, red) and long arm (q, green) of Aot-2 (the second largest chromosome in AAB) in mother, father, and hybrid offspring. (*A*) A metaphase spread of the AAB-ID25F mother. Tiny yellow bands (white bars) between the red and green colors indicate locations of common parts in both probes of 2p and 2q. (*B*) DAPI-G-band of the "*A*" picture. (*C*) A metaphase spread of the ALG-ID14M father. (*D*) DAPI-R-band of the "*C*" picture. (*E*) A metaphase spread of ID37F, the hybrid offspring of AAB-ID25F × ALG-ID14M. (*F*) DAPI-G-band of the "*E*" picture. (*G*) A metaphase spread of ID40F, another hybrid offspring of the same parents. (*H*) DAPI-G-band of the "*G*" picture. (*I*) Relationship between Aot-2 and -3. White markers in the black and white pictures highlight segments of characteristic chromosomes as follows: circle, the short arm (Aot-1p) and diamond, the long arm (Aot-1q) of Aot-1 (the largest chromosome in AAB, see also fig. 3); asterisk, the short arm (Aot-2p) of Aot-2; hollow triangles, the long arm (Aot-2q = Aot-3p) of Aot-2 (the second largest chromosome in AAB); triangle, the long arm (Aot-3q) of Aot-3 (the third largest chromosome in *Aotus*). X indicates the X chromosome. Black arrows in (*I*) indicate sequence of chromosome change. Black bar shows location of repeat sequences (the yellow bands described above). *fu*, fusion. *fi*, fission. For details see text. Scale bar = 10 μm.

suggests that Aot-2 was probably formed by centric fusion of the p (red) and q (green) arms, followed by an inversion between the two. Centric fission is not a feasible mechanism to explain the formation of Aot-2 because the acrocentric (2p, red small chromosome) lacks the yellow band at the proximal region. That is, both rearrangements—prior fusion and postpericentric inversion—are required for the yellow band formation at both the proximal regions. Aot-3 (2q (=3p) and 3q) involves both fusion and fission (fig. 4*I*).

Karyotypes of the Hybrid Offspring

As mentioned, the larger and more conspicuous chromosomes are useful markers for identifying night monkey hybrids. Of the four hybrids analyzed in this study, two showed "simple" mixed karyotypes with different chromosome numbers, $2n = 51$ (ID37F) and $2n = 52$ (ID39F). This difference stems from having two possible types of Aot-3, Aot-3pq and Aot-3p (= Aot-2q) (see fig. 4*C* and *D*). The formation of Aot-2 and Aot-3 are both demonstrated in figure 4*I*.

The two other hybrid monkeys, ID40F and ID41F, exhibited more complicated mixtures of karyotypes with de novo alterations of chromosomes. ID40F has 53 chromosomes that include an X-trisomy. Analysis with the OwlRep marker revealed that the probe bound to two of the X-chromosomes at the proximal region of the short arm, but showed no evidence of binding on the third (fig. 5). Thus, the trisomy comprises two X-chromosomes inherited from the mother (species AAB) and one X inherited from the father (species ALG) (figs. 4*H* and 5).

Hybrid ID41F had a karyotype similar to ID37F, but with some other extra chromosome alterations. The first of these was a trisomy of chromosome 18 that was eventually identified using a paint probe made from chimpanzee whole arm 6p + 6q (chimp6pq) and another made from the p arm of AAB chromosome 2 (Aot-2p). We inferred from DAPI-band analysis that chimpanzee 6p is homologous with the Aot-2p and chimpanzee 6q is homologous with the whole of AAB chromosome 18. The chimp6pq probe painted four small chromosomes and a short arm of the Aot-2 in hybrid ID41F (fig. 6*A*). We subsequently made a paint probe from the Aot-

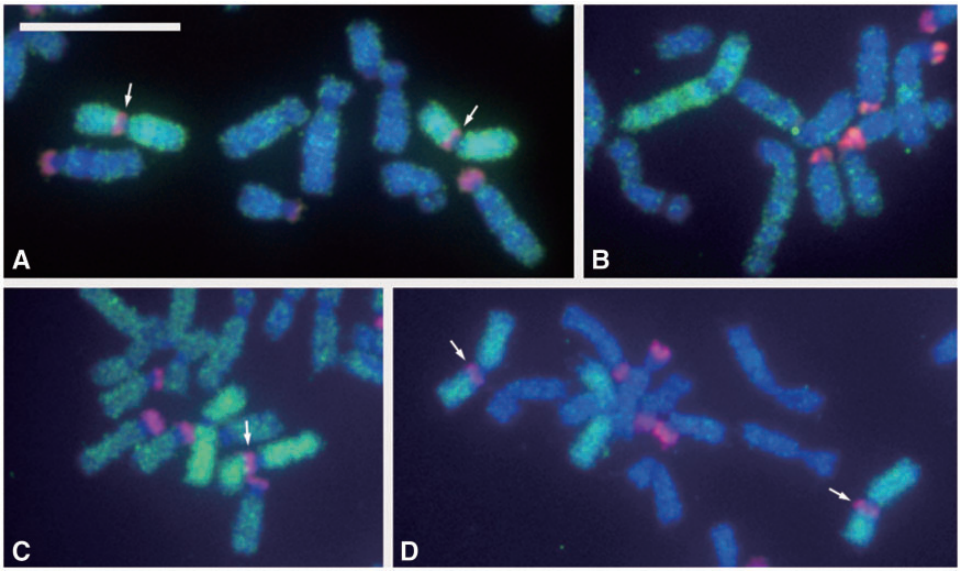

Fig. 5.—X chromosomes of AAB female (ID25F) (*A*), of ALG male (ID14M) (*B*), of hybrid with its disomy (ID37F) (*C*), and of hybrid with its trisomy (ID40F) (*D*). Green color highlights regions hybridizing to the whole X chromosome paint probe made from the X of ALG. Red color highlights regions hybridized with repetitive DNA (OwlRep). Notably, the X chromosome of AAB hybridizes with the OwlRep marker at the proximal region of the short arm, but that of ALG does not. White arrow indicates the location hybridized with the probe at the proximal region of the X chromosome. Scale bar = 10 μm.

2p (fig. 6*A*, white square bracket) and found that it, in turn, hybridized with only two segments (fig. 6*B*) of the five that bound the probe of chimp chr6 short arm (chimp6p). Thus, the three unstained segments (compare fig. 6*B* and *C*)—which corresponded to chimp chr6 long arm (chimp6q)—were confirmed to be a trisomy of the AAB chromosome 18 (fig. 6*C*). These trisomies of chromosomes X and 18 were observed as de novo formations only in the hybrids. Interestingly, both were found in the mother's genomic contribution to the hybrids, as indicated by the X + OwlRep-marker and the short arm heterochromatin length of chromosome 18, which is longer in the mother's (AAB) chromosome and shorter in father's (ALG) chromosome (fig. 6*C*). Another hybrid (ID37F) showed two disomies (yellow, chimpanzee 6p and red, chimpanzee 6q) of chromosome 18 in AAB and ALG (fig. 6*D*).

In addition to these trisomies, we found two different karyotype patterns with respect to chromosomes 21 and 23 in the preparations from the blood cells of hybrid ID41F (fig. 7). DAPI-band analysis revealed that both types were different from the standard pattern (fig. 7*A*, +, =). To examine these areas more closely, we made two paint probes from the chromosomal segments thought to be involved in the rearrangement, a green one from the terminal region of the larger acrocentric (fig. 7*A*, "+" with square bracket), and a red one from the long arm of the smaller metaphase chromosome (fig. 7*A*, "=" with square bracket). These probes hybridized with *three* different chromosomes on the ID41F plate: the two from which the probes were made (fig. 7*A*, +, =), and a smaller acrocentric, which hybridized with red and green (fig. 7*A*, arrowhead). The proximal segment of one of the dyed chromosomes (fig. 7*A*, "+" region) was similar to the bands of chromosome 21; to confirm it, we then painted with human paint probe (HAS17, green), because it is known to have homology with chromosome 21 in night monkeys (Ruiz-Herrera et al. 2005). The red and green paint probes produced in the present study (+, =) provided information on the inheritance of chromosome 23. That is, in this pair, the larger heterochromatic short arm is the maternal inheritance (derived from species AAB) and the smaller heterochromatic short arm is the paternal inheritance (derived from species ALG) (fig. 7*C* and *D*). Figure 7*D* shows the heterochromatin growth of the short arm, which is a whitish part of the DAPI-band of the chromosome marked with the asterisk in figure 7*C*. The HAS17 probe hybridized with three other regions on the same plate (fig. 7*B*, arrows), which are all segments of chromosome 21. The chromosomal patterns depicted in figure 7*A* and *B* thus allowed us to infer a pathway of rearrangements related to the four chromosomes: reciprocal translocation between chromosomes 21 and 23 (fig. 7*E*, right of bottom row). Specifically, the chromosome characteristics reveal that the translocation occurred between the mother's chromosomes 21 and 23.

Another interesting result was discovered in hybrid ID41F: a mosaic condition of these chromosome alterations exists between skin and blood cells. The skin cells showed only the chromosome 18-trisomy. However, the blood cells were of two types, where one type exhibited the 18-trisomy alone

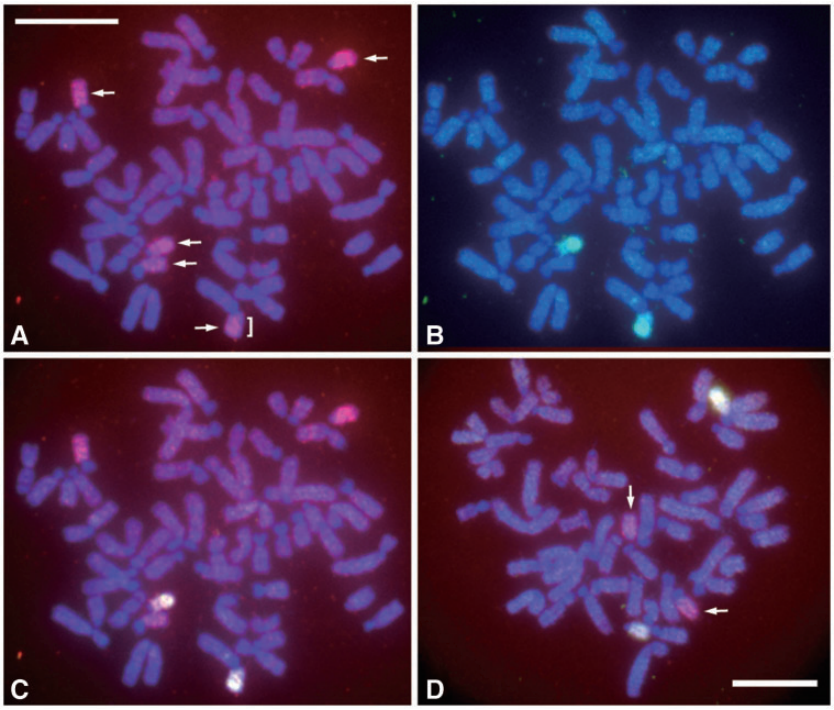

Fig. 6.—Painting analysis of autosome 18 trisomy. (*A*)–(*C*) A hybrid individual (ID41F) with the trisomy. (*D*) Another hybrid (ID37F) with the disomy. Paint probes made from chimpanzee 6p and 6q (red) hybridized with chromosome 18 of AAB. Paint probe made from the short arm of chromosome 2 (Aot-2p) (*A*, square bracket) of AAB enhanced sorting the five chromosomes painted by red. The probe hybridized to two chromosomes (Aot-2p) of the five (*B*, green; *C* and *D*, yellow), which correspond with chimpanzee 6p. Hence, the remnant three chromosomes indicate autosome 18 trisomy in ID41F (*C*, red) and disomy in ID37F (*D*, red)—and these segments correspond with chimpanzee 6q (18 of AAB). Arrowheads show mother's chromosome 18 with the longer short arm heterochromatin. White arrow indicates the chromosome hybridized with the chimpanzee 6pq probe (red). Scale bar = 10 μm.

and the other type exhibited this pattern in combination with the translocation between chromosomes 21 and 23, designated as mos52,XX,+18[87]/52,XX,+18,t(21;23)[85]. Here, each clone size (number of cells observed) of the mosaic karyotypes is depicted in square brackets.

Karyotypic constructions of the parents (AAB and ALG) and four hybrid individuals examined in the present study are summarized in figure 8.

Sex Ratio and Life Span of Interspecies Hybrid Offspring

Night monkey ID25F gave birth to seven interspecies hybrids (fig. 2), among which only one was male. Though the number of hybrid offspring are few, the skewed sex ratio in favor of females follows Haldane's (1922) rule: "When in the F1 offspring of two different races one sex is absent, rare, or sterile, that sex is the heterozygous sex." Only four females (ID37F, ID39F, ID40F, ID41F) were cytogenetically examined here because the other three individuals died before this study was begun. Hybrids ID40F and ID41F subsequently passed away at ages 19 and 23, respectively, which is consistent with the longevity of nonhybrid captive female night monkeys (Larson et al. 2016; Aquino and Encarnación 1994, and our KUPRI rearing records). The morphology and behavior of the monkeys with these chromosomal aberrations appeared normal.

Discussion

In the present study, we found two trisomies and a translocation in interspecies hybrids of night monkeys (fig. 8). Chromosome and DNA markers indicated that the X-chromosome and autosome 18 trisomies observed in the hybrids are derived from the mother's genome set, *A. azarae bolibiensis* (AAB ID25F). The two trisomies were found in all the cells observed of both blood and skin in the hybrid (ID41F), and thus must have been produced in the early embryo before the development of organs. Though the causative mechanism for such trisomies is unclear, nondisjunctions of chromosomes are postulated to have occurred in the mother's genome set during meiotic cell division postfertilization. Increased maternal age may play a role (Hassold and Jacobs 1984; Gaulden 1992; Nagaoka et al. 2012), and depletion of the cohesin that stabilizes the chromosome bivalent during aging is a leading hypothesis for the mechanism (Herbert et al. 2015). Though

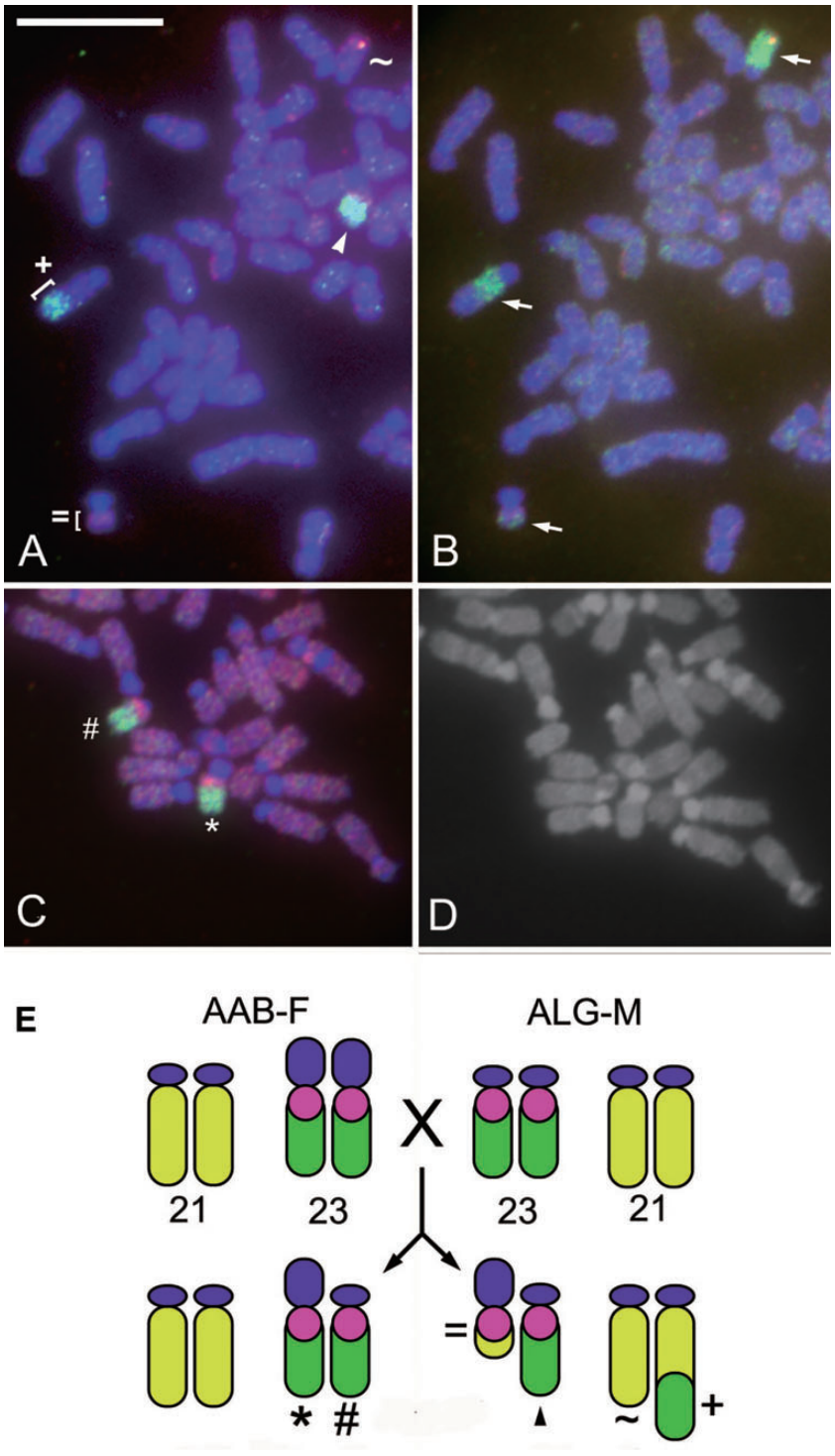

Fig. 7.—Reciprocal translocation found in blood cells of a hybrid ID41F. (*A*) Localization of two paint probes, green (+) and red (=), produced from the segment shown by square brackets. (*B*) Localization of a human chromosome paint probe (HAS17) homologous with night monkey chromosome 21 on the same chromosome plate that was washed following the procedure of figure "*A*". (*C*) Partial metaphase containing intact chromosome 23, showing the maternal (*) and paternal (#) inheritance. (*D*) DAPI-band of "*C*" metaphase showing heterochromatin as whitish part. (*E*) Schematic identification of translocation between chromosomes 21 and 23 (right of bottom row, markers (+, ~, triangle, =, *, #) are same as those of (*A*) and (*C*)) and simple mixture type of the two species (left of bottom row). AAB-F, female of *Aotus azarae boliviensis*, ALG-M, male of *Aotus lemurinus griseimembra*. Scale bar = 10 μm.

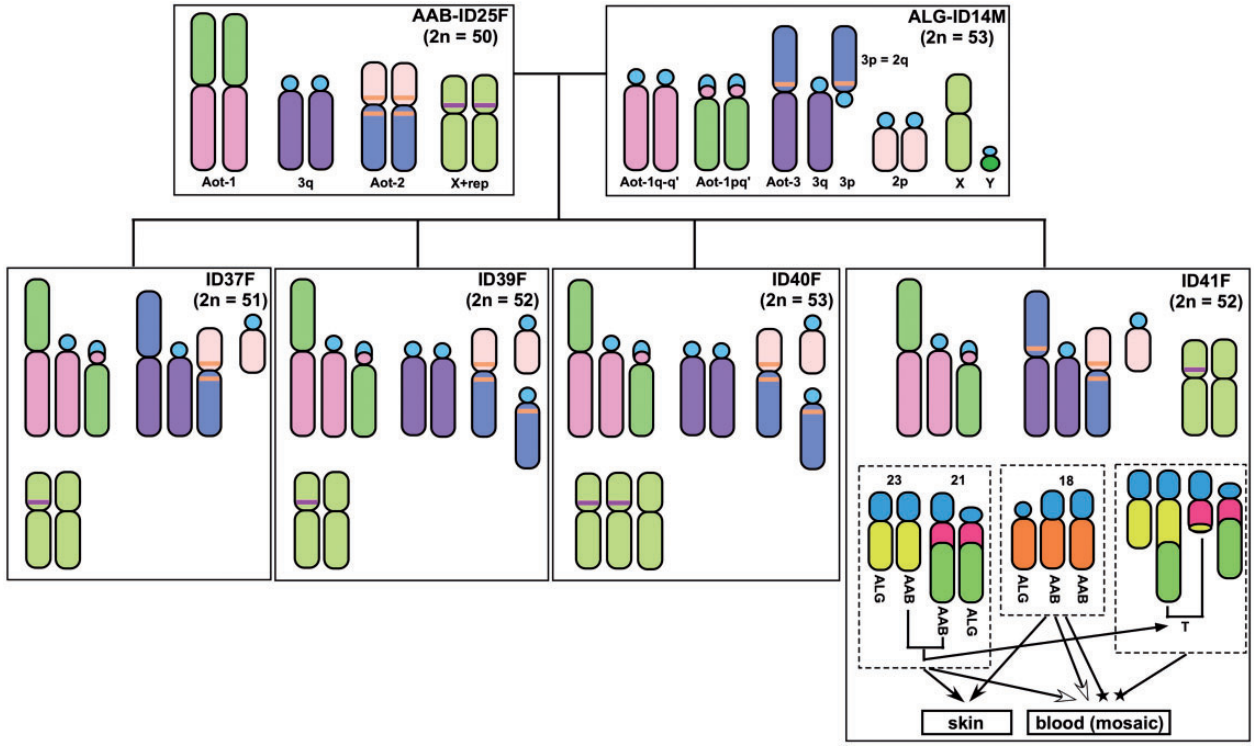

Fig. 8.—Summary of chromosome alterations in hybrid offspring between AAB and ALG. Chromosomes Aot-1, Aot-2, Aot-3, 18, 21, 23 and X are used as markers for identifying chromosome structures of parent and hybrid offspring. Specifically, this figure depicts the karyotype relationships among animals ID25F, ID14M, ID37F, ID39F, ID40F and ID41F at the Primate Research Institute, Kyoto University.

the trisomies in the present study were formed when the mother (ID25F) was 15 and 16-years-old, another female (ID34F, see fig. 2) gave birth to chromosomally normal *nonhybrid* offspring at the same ages. Beside a possible maternal age effect, it is postulated that the trisomies and translocation described here may have resulted from genome stress induced by hybridization in meiotic division of postfertilization, defined here as a "hybridization effect". Recently, it was observed that blastomeres in embryos with micronucleation were associated with reciprocal chromosomal gains and losses in humans; thus, the formation of extra-nuclear DNA may be a primary mechanism for occurrence of aneuploidies (Kort et al. 2016). We were not able to examine such DNA micro-alterations in the present study, yet there seems to be a high possibility of production of such DNA micro changes due to a sort of "molecular stress" in interspecies hybridization (hybridization effect).

Chromosomal instabilities have been observed in kangaroo and wallaby hybrids. In these marsupials, 5–7 chromosomal rearrangements were observed, and these appeared to stem from retroelement alterations related to genomic methylation modification (O'Neil et al. 1998, 1999, 2001). An altered genetic background including hybridization brings about genomic stress that may be the impetus for the activation of mobile elements that are normally "silent" (McClintock 1984). Sumner (2003) reports that transposable elements accumulate in heterochromatin and appear to have an influential role in chromosome structure and function.

Additionally, Rieseberg (2001) and Ostberg et al. (2013) described in sunflower and trout fish hybrids, respectively, which chromosome rearrangements suppressed recombination and, further, how recombination suppression restricts gene flow. Nonrecombined chromosomes impede genetic exchange and persist within admixed populations.

In the present study, the night monkey trisomies might result from nondisjunction in meiotic division of the hybrid early embryo (King 1993), and the translocation might be mediated by transposable elements (Gray 2000). Hybridization is therefore not always a dead end, but a potential source of a new array of genetic/chromosomal combinations that may ultimately give rise to new species (Fontdevila 2005). Taken together, genome stress induced by hybridization may activate chromosome rearrangements and may mediate speciation through gene flow reduction and meiotic drive in admixed populations. Though the chromosomal alterations induced by hybridization were observed in captive night monkeys, these results allow us to speculate that if similar hybridization events occur among wild populations, such

events may play a significant role in reticulate speciation and evolution of these primates.

Additionally, we perhaps need to consider the nature of embryogenesis in night monkeys. Though the sample number analyzed in the present study is small, our survey suggests that intraspecific breeding may be associated with a higher incidence of spontaneous abortion and stillbirth than interspecific breeding. We need to examine in more detail the factors that predispose birth disorder in night monkeys. However, if the birthing characteristics of the animals in our study are typical of night monkeys, it suggests that admixed populations may play a significant role in hybrid chromosome differentiation and hybrid speciation, as has been argued in plants and animals (e.g., Rieseberg 2001; Ostberg et al. 2013). These may prove to be important conditions for reticulate speciation of night monkeys, because mixed genomes and differentiated chromosomes induced by a "hybridization effect" can be maintained in the population.

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